

WHAT IS CLAIMED IS:

1. A method of characterizing the biological activity of a candidate compound comprising:

5 exposing one or more cells to said compound;

repetitively exposing said one or more cells to one or more electric fields so as to effect a controlled change in transmembrane potential of said one or more cells; and

monitoring, without using a patch clamp, changes in the transmembrane potential of said one or more cells.

10 2. The method of Claim 1, wherein said monitoring comprises detecting fluorescence emission from an area of observation containing said one or more cells.

3. The method of Claim 1, wherein said electric fields are biphasic.

4. The method of Claim 3, additionally comprising limiting spatial variation in electric field intensity so as to minimize irreversible cell electroporation.

15 5. The method of Claim 1, wherein one or more electrical fields cause an ion channel of interest to cycle between different voltage dependent states.

6. The method of Claim 5, wherein said one or more electrical fields cause an ion channel of interest to open.

20 7. The method of Claim 5, wherein said one or more electrical fields cause an ion channel of interest to be released from inactivation.

8. The method of Claim 1, wherein said one or more cells comprise a voltage sensor selected from the group consisting of a FRET based voltage sensor, an electrochromic transmembrane potential dye, a transmembrane potential redistribution dye, an ion sensitive fluorescent or luminescent molecule and a radioactive ion.

25 9. The method of Claim 1, wherein said one or more cells comprise a voltage regulated ion channel.

10. The method of Claim 9, wherein said voltage regulated ion channel is selected from the group consisting of a potassium channel, a calcium channel, a chloride channel and a sodium channel.

11. The method of Claim 1, wherein said electric field exhibits limited spatial variation in intensity in the area of observation of less than about 25% from a mean intensity in that area.

5 12. The method of Claim 11, wherein said one or more electrical fields varies over an area of observation by no more than about 15 % from the mean electrical field at any one time.

13. The method of Claim 12, wherein said one or more electrical fields varies over an area of observation by no more than about 5 % from the mean electrical field at any one time.

10 14. The method of Claim 1, wherein said one or more electrical fields comprises stimulation with either a square wave-form, a sinusoidal wave-form or a saw tooth wave-form.

15 15. The method of Claim 1, wherein said one or more electrical fields have an amplitude within the range of about 10 V/cm to about 100 V/cm.

16. The method of Claim 15, wherein said one or more electrical fields have an amplitude within the range of about 20 V/cm to about 80 V/cm.

17. The method of Claim 1, wherein said one or more electrical fields are repeated at a frequency of stimulation that is greater than or equal to the reciprocal of the transmembrane time constant of said one or more cells.

20 18. The method of Claim 1, wherein said one or more electrical fields are repeated at a frequency of stimulation within the range of zero to 1kHz.

19. The method of Claim 1, wherein said one or more electrical fields have a pulse duration within the range of about 100 microseconds to about 20 milliseconds.

25 20. The method of Claim 1, wherein said transmembrane potential is developed across the plasma membrane of said one or more cells.

21. A method of assaying the biochemical activity of a compound against a target ion channel comprising:

selecting a cell line having a normal resting transmembrane potential corresponding to a selected voltage dependent state of said target ion channel;

expressing said target ion channel in a population of cells of said selected cell line;

30 *Part B27*

exposing said population of cells to said compound;

repetitively exposing said population of cells to one or more electric fields so as to effect a controlled change in transmembrane potential of said one or more cells; and

5 monitoring changes in the transmembrane potential of said one or more cells.

22. The method of Claim 21, wherein said target ion channel is exogenously expressed in said cell line.

10 23. The method of Claim 21, wherein said cell line is transfected with nucleic acid encoding said target ion channel.

*Part B27*  
24. The method of Claim 23, wherein said cell line expresses insignificant levels of other ion channels.

25. The method of Claim 24, wherein said cell line is selected from the group consisting of CHL, LTK(-), and CHO-K1.

15 26. The method of Claim 21 wherein said target ion channel is a sodium channel, and wherein said population of cells is selected from the group consisting of CHL cells, LTK(-) cells, and CHO-K1 cells.

20 27. The method of Claim 21 wherein said target ion channel is a sodium channel, and wherein said population of cells is selected from the group consisting of HEK-293 cells, RBL cells, F11 cells, and HL5 cells.

28. The method of Claim 21 wherein said target ion channel is a potassium channel, and wherein said population of cells is selected from the group consisting of CHL cells, LTK(-) cells, and CHO-K1 cells.

25 29. The method of Claim 21 wherein said target ion channel is a calcium channel, and wherein said population of cells is selected from the group consisting of CHL cells, LTK(-) cells, and CHO-K1 cells.

30 A method of assaying ion channel activity comprising:  
exposing at least one cell to a plurality of electric field pulses so as to create a controlled change in transmembrane potential and so as to activate an ion channel of interest, and

detecting ion channel activity by detecting one or more changes in transmembrane potential without using a patch clamp.

31. The method of claim 30, wherein said at least one cell comprises a voltage sensor selected from the group consisting of a FRET based voltage sensor, an electrochromic transmembrane potential dye, a transmembrane potential redistribution dye, an ion sensitive fluorescent or luminescent molecule and a radioactive ion.

32. The method of Claim 31 wherein said voltage sensor comprises a FRET based voltage sensor.

33. The method of Claim 32, wherein said ion channel of interest is a voltage regulated ion channel.

34. The method of Claim 33, wherein said plurality of electric field pulses cause said ion channel of interest to cycle between different voltage dependent states.

35. The method of Claim 30, wherein said at least one cell is an eukaryotic cell.

36. The method of Claim 30, wherein said at least one cell is a non-excitable cell.

37. The method of Claim 30, wherein said at least one cell is a prokaryotic cell.

38. The method of Claim 30, wherein said at least one cell is a tissue culture cell.

39. The method of Claim 30, wherein said at least one cell is a primary cell line.

40. The method of Claim 30, wherein said at least one cell is part of an intact living organism.

41. A method of assaying ion channel activity comprising:  
expressing a selected target ion channel in at least one cell;  
expressing a selected counter ion channel in said at least one cell;  
exposing said at least one cell to a plurality of electric field pulses so as to create a controlled change in transmembrane potential and so as to activate said counter ion channel; and

monitoring the transmembrane potential of said at least one cell.

42. The method of Claim 41, wherein a transmembrane potential change is detected when said ion channel of interest is blocked.

43. The method of Claim 42, wherein said ion channel of interest comprises a ligand gated ion channel.

5 44. The method of Claim 43, wherein said counter channel comprises a sodium channel.

10 45. A method of modifying the transmembrane potential of a cell comprising repetitively applying biphasic electric field pulses to said cell, wherein said pulses have a maximum amplitude of less than approximately 90 V/cm, wherein said pulses are applied at a rate of at least about 1 per second, and wherein the total duration of each pulse is at least about 1 millisecond.

46. The method of Claim 45, wherein said maximum amplitude is approximately 20 to 40 V/cm.

15 47. The method of Claim 45, wherein said pulse duration is approximately 2 to 10 milliseconds per phase.

20 48. The method of Claim 45, wherein said pulses are applied at a rate of approximately 20 to 100 pulses per second.